

Seroprevalence of *Toxoplasma gondii* antibodies in wild carnivores from Spain

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Abstract

Serum samples from 282 wild carnivores from different regions of Spain were tested for antibodies to *Toxoplasma gondii* by the modified agglutination test using a cut-off value of 1:25. Antibodies to *T. gondii* were found in 22 of 27 (81.5%) of Iberian lynx (*Lynx pardinus*), 3 of 6 European wildcats (*Felis silvestris*), 66 of 102 (64.7%) red foxes (*Vulpes vulpes*), 15 of 32 (46.9%) wolves (*Canis lupus*), 26 of 37 (70.3%) Eurasian badgers (*Meles meles*), 17 of 20 (85.0%) stone martens (*Martes foina*), 4 of 4 pine martens (*Martes martes*), 6 of 6 Eurasian otters (*Lutra lutra*), 4 of 4 polecats (*Mustela putorius*), 1 of 1 ferret (*Mustela putorius furo*), 13 of 21 (61.9%) European genets (*Genetta genetta*), and 13 of 22 (59.1%) Egyptian mongooses (*Herpestes ichneumon*). Serological results indicated a widespread exposure to *T. gondii* among wild carnivores in Spain. The high *T. gondii* seroprevalence in Iberian lynx and the European wildcat reported here may be of epidemiologic significance because seropositive cats might have shed oocysts.

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1. Introduction

Toxoplasma gondii is an intracellular protozoan of worldwide distribution. Domestic and free-ranging felids are the only definitive hosts for *T. gondii*. In infected cats, oocysts are produced and shed in the feces. The definitive and intermediate host may be infected by ingestion of water or food contaminated

with oocysts, by ingestion of tissue cysts or by vertical transmission (Dubey and Beattie, 1988).

Virtually all warm-blooded hosts can be intermediate hosts of *T. gondii*. Surveys of *T. gondii* infection in free-ranging animals can provide estimates of environmental contamination and circulation of *T. gondii* in domestic and wild ecosystems. There are only a few surveys of *T. gondii* infection in wild animals in Spain and these include a report in wild rabbits (Almería et al., 2004), wild ruminants (Gauss et al., 2006), wild boar (Gauss et al., 2005), and wild cetaceans (Cabezón et al., 2004). In the present paper, we report seroprevalence of *T. gondii* in wild carnivores from

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Spain for the first time, particularly in an endangered species, the Iberian lynx (Table 1). We have also attempted to provide information on possible risk factors such as sex, age, and geographic origin of some of the animals.

2. Materials and methods

2.1. Source of animals

Serum samples were collected from 1990 to 2006, from a total of 282 legally obtained wild carnivores (Table 1) surveyed post-mortem (mostly road kills, except some fox samples obtained from hunters) or in vivo (from captures with scientific purposes). With the exception of the fox, all the sampled species are protected by Spanish law. The samples were from six Spanish regions (CC, Cantabric Coastal region; AR, Aragón; CL, Castile and León; SC, Central Spain; CV, Valencia Community; and SH, Seville-Huelva) that are representative of different bioregions of the Iberian Peninsula (Fig. 1). Age was determined by animal size and teeth characteristics (Saénz de Buruaga et al., 1991). Sex and geographic origin was recorded whenever possible. Blood samples were collected from the heart or chest cavity of dead animals and from the cephalic vein of live animals and sera were stored at -20°C until analysis was performed.

2.2. Serological examination

The prevalence of antibodies to *T. gondii* was determined by the modified agglutination test (MAT) as described previously (Dubey and Desmonts, 1987) with minor modifications. To eliminate particulate matter (erythrocytes, bacteria), samples were filtrated using a sterile $0.2\text{-}\mu\text{m}$ microfilter (Nalgene®). Hemolysis of sera generally is not a major problem for the detection of *T. gondii* antibodies with the MAT using a 1:25 dilution of serum and mouse-derived tachyzoites (Dubey et al., 2003). Each serum sample was tested at dilutions of 1:25, 1:50, 1:100, and 1:500. A commercial positive control serum (Toxotrol A, Biomerieux, France) diluted from 1:25 to 1:3200 (with a minimum titer of 1:200 in each test) and serum dilution buffer without serum as negative control were included in each test. Sera with a titer of 1:25 or higher were considered positive and those with doubtful results were re-tested. Filtration had no interference with titration of the positive control serum used.

MAT has been validated in wild carnivores such as bobcats, raccoons, coyotes, gray and red foxes, and black bears based on parasite isolation and MAT seropositivity (Dubey et al., 2004). Although the specificity and sensitivity of MAT have not been evaluated for the diagnosis of toxoplasmosis in many other wildlife species, it is the most sensitive and specific test for the diagnosis of toxoplasmosis in

Table 1
Seroprevalence of *T. gondii* in wild carnivores from Spain

Family	Species	Regions*	Number tested	Number positive (%)	Number with antibody titres of			
					1:25	1:50	1:100	1:500
Felidae	Iberian lynx (<i>Lynx pardinus</i>)	SC; SH ^a	27	22 (81.5)	1	3	8	10
	European wildcat (<i>Felis silvestris</i>)	CC; SC	6	3 (50.0)	0	0	1	2
Canidae	Red fox (<i>Vulpes vulpes</i>)	CC; CL; SC; SH; AR ^b	102	66 (64.7)	21	10	22	13
	Wolf (<i>Canis lupus</i>)	CC; CL	32	15 (46.9)	11	4	0	0
Mustelidae	Eurasian badger (<i>Meles meles</i>)	CC; SC; SH ^c	37	26 (70.3)	12	4	6	4
	Stone marten (<i>Martes foina</i>)	CC; SC; CV ^d	20	17 (85.0)	5	7	2	3
	Pine marten (<i>Martes martes</i>)	CC	4	4 (100)	2	0	0	2
	Ferret (<i>Mustela putorius furo</i>)	CC	1	1 (100)	1	0	0	0
	Eurasian otter otters (<i>Lutra lutra</i>)	CC; SC ^e	6	6 (100)	3	3	0	0
	Polecat (<i>Mustela putorius</i>)	CC; SC	4	4 (100)	0	1	2	1
Viverridae	European genet (<i>Genetta genetta</i>)	CC; SC; SH; CV	21	13 (61.9)	2	2	7	2
Herpestidae	Egyptian mongoose (<i>Herpestes ichneumon</i>)	SC; SH	22	13 (59.1)	6	1	6	0

* CC, Cantabric Coastal region; AR, Aragón; CL, Castile and León; SC, Central Spain; CV, Valencia Community; and SH, Seville-Huelva.

^a Two samples without location.

^b Four samples without location.

^c Three samples without location.

^d Four samples without location.

^e One samples without location.

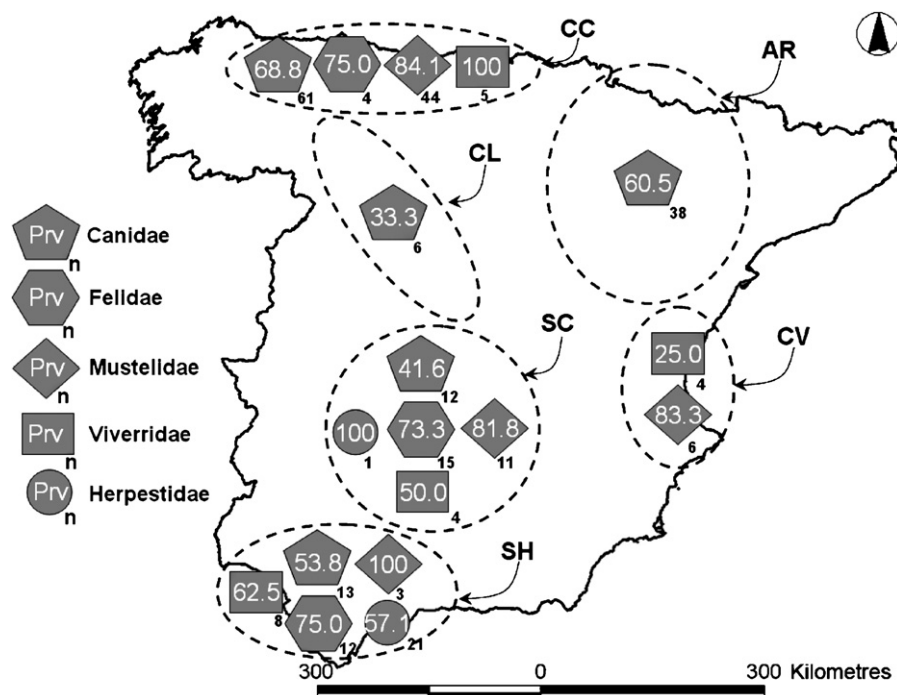


Fig. 1. Map of peninsular Spain showing the prevalence of antibodies against *T. gondii* in five carnivore families (inside the polygons), and the sample size (as subscripts). CC, Cantabric Coastal region; AR, Aragón; CL, Castile and León; SC, Central Spain; CV, Valencia Community; and SH, Seville-Huelva.

animals, particularly pigs (Dubey et al., 1995; Dubey, 1997). A titer of $\geq 1:25$ was considered indicative of *T. gondii* infection in wild carnivores as used for other species (Dubey and Beattie, 1988; Dubey et al., 2003).

2.3. Statistical analysis

Seroprevalence was statistically analyzed considering the variables of geographical area, year of sample collection, sex, age of the animals and taxonomic family. The statistical data analysis was performed using the SPSS 14.0 Statistical program by χ^2 test. The differences between variables were analyzed by Bonferroni or Tukey–Kramer tests. For multiple comparison Dunn's test was performed and when variances were not homogenous, non-parametric test were performed. The differences were considered statistically significant when $p \leq 0.05$.

3. Results

Antibodies (MAT 1:25 or higher) against *T. gondii* were detected in 190 (67.4%) of 282 animals (Table 1). Antibodies were observed in all the surveyed species.

There were no significant differences in *T. gondii* seroprevalence between males and females considering species, taxonomic families and all analyzed samples together (data not shown).

When considering each individual species, no statistically significant differences in *T. gondii* seroprevalence with age were observed. Age-related differences in seroprevalence were observed with a significantly higher seroprevalence in adults ($p < 0.01$) when all the analyzed samples were considered together. Antibodies were detected in 116 (72.5%) of 160 adults and 20 (48.8%) of 41 juveniles. No data were available from 81 samples. No statistically significant differences were observed in age by taxonomic family.

The prevalence of *T. gondii* in mustelids was significantly higher than in the canids ($p < 0.01$), but there was no significant difference with other groups of animals. In the Cantabric Coast region, where large numbers of samples for both mustelids and canids were available, the former, although higher, showed no significant difference in the prevalence of antibodies to *T. gondii* (84.1%) as compared to the latter (68.8%, $p > 0.05$).

Considering the year of sample collection of foxes from 1990 to 2006, there were no significant differences in the prevalence among years.

4. Discussion

Seroprevalence of *T. gondii* in wild carnivores (mean 67.4%) from Spain was higher compared to the omnivorous wild boar (36% of 507, Gauss et al., 2005), herbivorous ruminants (10–33%, Gauss et al., 2006) or wild rabbits (14.2% of 456, Almería et al., 2004). These results are in agreement with those of Smith and Frenkel (1995) and Hejlíček et al. (1997) who hypothesized that due to the feeding habits of the species, it is more probable that carnivores have higher prevalence of antibodies against *T. gondii* due to accumulative ingestion of infected meat from other animals, and successively the prevalences are lower in omnivorous, herbivorous, and insectivorous mammals.

There are multiple reports of *T. gondii* seroprevalence in red foxes, with recent reports of seroprevalence of 56% of 206 red foxes by indirect immunofluorescence test (IFAT) in rural Ireland (Murphy et al., 2007) or 68% of 337 red foxes by direct agglutination test in Hungary (Jakubek et al., 2007). Similar seroprevalence levels (64.7% of 102 red foxes) were observed in our study. Higher seroprevalence levels were observed in red foxes in USA (85.9%) by MAT (Dubey et al., 1999).

With the exception of red foxes, there are few surveys of seroprevalence of *T. gondii* in other wild carnivores. Seroprevalence (46.9% of 32) in wolves (*Canis lupus*) in the present study was higher than that in Alaska, USA (9% of 125) (Zarnke et al., 2000); MAT was used in both studies. Using an IFAT, Sedlák and Bártová (2006) found antibodies in two of 10 captive wolves in Czech Republic. Using an ELISA, Philippa et al. (2004) did not find *T. gondii* antibodies in nine wolves from Canada.

In Mustelidae, Hejlíček et al. (1997) in the Czech Republic, based on isolation, observed the presence of *T. gondii* in 1 of 4 (25%) weasels (*Mustela nivalis*), in 1 of 3 (33%) European polecats (*M. putorius*), in 2 of 11 (18%) stone martens (*Martes foina*), and in 1 of 6 (17%) pine martens (*Martes martes*). More recently, DNA of *T. gondii* has been observed in the brain of 4.9% stone martens of 61 pine and stone martens (Hůrková and Modrý, 2006). Although, our results showed higher seroprevalence of *T. gondii* in European polecats (*M. putorius*), stone martens (*Martes foina*), and pine martens (*Martes martes*) compared to the study of Hejlíček et al. (1997), different techniques were performed, and therefore results are not comparable. Among mustelids, *T. gondii* antibodies had also been reported in black-footed ferrets (*Mustela nigripes*), farmed mink (*Mustela vison*), river otters (*Lontra canadensis*), and southern sea otters (*Enhydra lutris*

neris) (reviewed by Anwar et al., 2006). Congenital toxoplasmosis was reported in ferrets (*Mustela putorius furo*) in New Zealand (Thornton and Cook, 1986).

Recently, Anwar et al. (2006) reported for the first time *T. gondii* seroprevalence of 70% in 90 Eurasian badgers in England by the latex agglutination test; this prevalence is almost identical to (70.3%) prevalence in the present study, although using different techniques.

We report here for the first time in Spain the presence of *T. gondii* antibodies in Eurasian otters (*Lutra lutra*). Until recently, water-borne transmission of *T. gondii* was considered uncommon, but the widespread infection of marine mammals in the USA and elsewhere provide reasons to question this view (Dubey, 2004; Cabezón et al., 2004) and river water contamination could be the main reason for the observed *T. gondii* infection of Eurasian otters in our study. To our knowledge, this is also the first report of *T. gondii* seroprevalence in the Egyptian mongoose (*Herpestes ichneumon*) and in the genet (*Genetta genetta*).

Seroprevalence in wild felids in the present study (81.5% in Iberian lynx and 50% in European wildcat) was higher than that observed in domestic cats from Spain (45%, Gauss et al., 2003; 32.3%, Miró et al., 2004). This can be explained considering the different diets of both groups of hosts. The main diet for wild felids in Spain, particularly Iberian lynx, is wild rabbits. Iberian lynx diet consists 85% of wild rabbits (Gil-Sánchez et al., 2006) and *T. gondii* seroprevalence in wild rabbits has been found to be high (up to 53.8%) in some areas of Spain (Almería et al., 2004).

The high seroprevalence of *T. gondii* observed in the Iberian lynx is of historic and biologic significance. Antibodies to *T. gondii* in other lynx species such as Eurasian lynx (*Lynx lynx*) were reported in 75.4% of 207 lynx from Sweden (Ryser-Degiorgis et al., 2006), and 73% of 70 from Norway (Oksanen and Lindgren, 1995). Other species of lynx in North America, bobcat (*L. rufus*) or the Canada lynx (*Lynx canadensis*) were also reported to have high levels of MAT antibodies (Labelle et al., 2001; Mucker et al., 2006 and references contained). Additionally, experimentally-infected bobcats shed *T. gondii* oocysts (Miller et al., 1972). An acute outbreak of toxoplasmosis in humans in Canada was epidemiologically linked to contamination of a water reservoir with oocysts excreted by cougars, *Felis concolor vancouverensis* (now *Puma concolor*) (Aramini et al., 1998; Bowie et al., 1997). In the present study, three of six *Felis silvestris* had antibodies to *T. gondii*. Few studies have been performed in this species. In Great Britain, *T. gondii* antibodies were detected in 100% of 23 wildcats tested (McOrist, 1992) and

Lukešová and Literák (1998) identified *T. gondii* oocysts in three wildcats living in a zoo in the Czech Republic. Although oocyst excretion by Iberian lynx has not been reported to date, it is likely that all felids shed *T. gondii* in nature and they are important in maintaining the sylvatic cycle of this parasite.

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